U.S. DEPARTMENT OF COMMERCE PATENT & TRADEMARK OFFICE

B/O Form PTO-1390 **Transmittal Letter to the United States Designated/Elected Office (DO/EO/US) Concerning a Filing Under *35 USC 371***		*Attorney's Docket Number* HOGL3001/REF
		U.S. Application Number (if known) 10/070412
International Application Number PCT/SE00/01767	*International Filing Date* September 13, 2000	*Priority Date Claimed* September 13, 1999
Title of Invention DNA CONSTRUCT AND ITS USE		
Applicant(s) for DO/EO/US HOGLUND et al.		

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items under *35 USC 371*:

1. ☒ This is a **FIRST** submission of items concerning a filing under *35 USC 371*.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under *35 USC 371*.
3. ☒ This express request to begin national examination procedures *(35 USC 371(f))* at any time rather than delay examination until the expiration of the applicable time limit set in *35 USC 371(b)* and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed *35 USC 371(c)(2)*.
   - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☒ has been transmitted by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English *(35 USC 371(c)(2))*.
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3))
   - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☐ have been transmitted by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 *(35 USC 371(c)(3))*.
9. ☒ An oath or declaration of the inventor(s) *(35 USC 371(c)(4))*. (☒ Executed ☐ Unexecuted)
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 *(35 USC 371(c)(5))*.

Items 11 to 16 below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under *37 CFR 1.97* and *1.98*.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with *37 CFR 3.28* and *3.31* is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English translation of the international application under 35 U.S.C. 154(d)(4)
20. ☒ Other items or information: Sequence Listing and Application Data Sheet

Application Number (if Known)	International Application Number	Attorney's Docket Number
10/070412	PCT/SE00/01767	HOGL3001/REF

	Calculations	PTO USE ONLY
1. The following fees are submitted:		
Basic National Fee *(37 CFR 1.492(a)(1)-(5))*:		
☒ Neither International Preliminary Examination Fee *(37 CFR 1.482)* nor International Search Fee *(37 CFR 1.445(a)(2))* paid to USPTO $1040.00		
☐ Search report has been prepared by the EPO or JPO $890.00		
☐ International Preliminary Examination Fee paid to USPTO *(37 CFR 1.482)* $710.00		
☐ No International Preliminary Examination Fee paid to USPTO *(37 CFR 1.482)* but International Search Fee paid to USPTO *(37 CFR 1.445(a)(2))* $740.00		
☐ International Preliminary Examination Fee paid to USPTO *(37 CFR 1 482)* and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT	**$ 1,040.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date *(37 CFR 1.492(e))*.		

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE		
Total Claims	20	-20 =	0	× $18.00	$ 0.00	
Independent Claims	1	-3 =	0	× $84.00	$ 0.00	
Multiple Dependent Claims (if applicable)				+ $280.00		
TOTAL OF ABOVE CALCULATIONS						
Reduction by ½ for filing by small entity, if applicable. Small Entity Status is asserted pursuant to 37 CFR 1.27 for this application.					$ 520.00	
SUBTOTAL					**$ 520.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date *(37 CFR 1.492(f))*.						
TOTAL NATIONAL FEE						
Fee for recording the enclosed assignment *(37 CFR 1.21(h))*. The assignment must be accompanied by an appropriate cover sheet *(37 CFR 3.28, 3.31)*. **$40.00** per property.					$ 40.00	
TOTAL FEES ENCLOSED					**$ 560.00**	
Amount to be:					Refunded:	
					Charged:	

a. ☒ A check in the amount of **$560.00** to cover the fees is enclosed.

b. ☐ Please charge my **Deposit Account Number 02-0200** in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to **Deposit Account Number 02-0200**. A duplicate copy of this sheet is enclosed.

Note: Where an appropriate time limit under *37 CFR 1.494* or *1.495* has not been met, a petition to revive *(37 CFR 1.137(a) or (b))* must be filed and granted to restore the application to pending status.

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23364
PATENT TRADEMARK OFFICE

DATE: March 15, 2002

Respectfully submitted,

Richard E. Fichter

Attorney for Applicant: Richard E. Fichter
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#4

STATEMENT

I, Brita Nilsson, hereby certify that the information recorded in computer readable form is identical to the written sequence listing as filed with the original PCT application PCT/SE00/01767.

Signed

Brita Nilsson

April 10, 2002

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

HOGLUND et al.

U.S. National Phase of PCT/SE00/01767

Entry papers filed herewith March 15, 2002

For: DNA CONSTRUCT AND ITS USE

Attention: PCT OFFICE

PRELIMINARY AMENDMENT
AND INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The present application is the U.S. national phase of international application number PCT/SE00/01767. The following amendments pertain to the claims as amended.

Please note that the amended page 1 (new claim set) attached to the International Preliminary Examination Report (Annexes) and submitted herewith, have replaced the originally filed page 8 of the application. The claims to be examined and amended by this preliminary amendment are found on amended page 1 (of the new claim set).

Please amend the above-identified application as follows:

IN THE SPECIFICATION:

Please add the attached ABSTRACT OF THE DISCLOSURE to the application.

IN THE CLAIMS:

Please replace claims 3 and 5-6 with the following amended claims.

3(Amended). Transgenic oilseed plant cell according to claim 1, wherein the promoter is a napin promoter, the peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification is selected from the group consisting of peptides with, 1-D-deoxyxylulose 5-phosphate synthase, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, zeta-carotene desaturase, lycopene beta-cyclase, β-carotene hydroxylase, and acyl transferase activity.

5(Amended). Transgenic oilseed plant cell according to claim 1, wherein the oilseed plant is selected from the group consisting of rape, sunflower, soybean and mustard.

6(Amended). Transgenic oilseed plant cell according to claim 1, wherein the cell expresses xanthophyll.

Please add the following new claims to the application.

10(New). Transgenic oilseed plant cell according to claim 2, wherein the promoter is a napin promoter, the peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification is selected from the group consisting of peptides with, 1-D-deoxyxylulose 5-phosphate synthase, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, zeta-carotene desaturase, lycopene beta-cyclase, β-carotene hydroxylase, and acyl transferase activity.

11(New). Transgenic oilseed plant cell according to claim 2, wherein the oilseed plant is selected from the group consisting of rape, sunflower, soybean and mustard.

12(New). Transgenic oilseed plant cell according to claim 3, wherein the oilseed plant is selected from the group consisting of rape, sunflower, soybean and mustard.

13(New). Transgenic oilseed plant cell according to claim 4, wherein the oilseed plant is selected from the group consisting of rape, sunflower, soybean and mustard.

14(New). Transgenic oilseed plant cell according to claim 10, wherein the oilseed plant is selected from the group consisting of rape, sunflower, soybean and mustard.

15(New). Transgenic oilseed plant cell according to claim 2, wherein the cell expresses xanthophylls.

16(New). Transgenic oilseed plant cell according to claim 3, wherein the cell expresses xanthophylls.

17(New). Transgenic oilseed plant cell according to claim 4, wherein the cell expresses xanthophylls.

18(New). Transgenic oilseed plant cell according to claim 5, wherein the cell expresses xanthophylls.

19(New). Transgenic oilseed plant cell according to claim 10, wherein the cell expresses xanthophylls.

20(New). Transgenic oilseed plant cell according to claim 14, wherein the cell expresses xanthophylls.

REMARKS

Applicants have amended the claims in order to reduce the initial filing fee by deleting the multiple dependent claims from the application. Some of this subject matter has been reintroduced as dependent claims 10-20. Applicants retain the right to reintroduce any subject matter canceled by the present Amendment at any time during the prosecution of this application or any further application claiming benefit of this application.

Applicants have amended the application to substitute the originally filed page 8 with the amended claim set page 1 attached to the International Preliminary Examiner Report (Annexes) and included in the application as filed herewith. Also, an Abstract of the Disclosure has been added to the application.

Applicants are submitting herewith a copy of the International Search Report which issued on International Application No. PCT/SE00/01767, of which the present application is the U.S. national phase which was published in English. All of the publications cited in the International Search Report are listed on the attached Form PTO-1449. It is Applicants' understanding that, under the procedures of the PCT, copies of the cited publications will have been supplied to the U.S. Patent Office by the International Bureau. However, the Examiner is invited to contact the undersigned attorney if additional copies are necessary or would facilitate examination of the present application.

Otherwise, the Examiner is respectfully requested to return an initialed and dated copy of the attached Form PTO-1449 to confirm that all publications listed thereon have been considered and made officially of record in the file of this application.

Applicants understand that, under the procedures of the PCT, a copy of the priority document (SE 9903336-7, filed 17 September 1999) will have been supplied to

the U.S. Patent Office pursuant to Rule 17 of the PCT Regulations. It is therefore respectfully requested that the first Official Action in the present application contain an indication that the appropriate priority document is in the file of this application.

In view of the above amendments, an early action on the application is now in order and is most respectfully requested.

Respectfully submitted,
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DATE: March 15, 2002

Marked-Up Version Showing Changes Made

IN THE CLAIMS:

Please replace claims 3 and 5-6 with the following amended claims.

3(Amended). Transgenic oilseed plant cell according to claim 1 [or 2], wherein the promoter is a napin promoter, the peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification is selected from the group consisting of peptides with, 1-D-deoxyxylulose 5-phosphate synthase, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, zeta-carotene desaturase, lycopene beta-cyclase, β-carotene hydroxylase, and acyl transferase activity.

5(Amended). Transgenic oilseed plant cell according to [any one of claims 1 - 5] claim 1, wherein the oilseed plant is selected from the group consisting of rape, sunflower, soybean and mustard.

6(Amended). Transgenic oilseed plant cell according to [any one of claims 1 - 5] claim 1, wherein the cell expresses xanthophyll.

29295/BN

Abstract

A DNA construct comprising in the 5′ to 3′ direction of transcription operably linked a promoter region directing transcription to the seed of an oilseed plant, a nucleotide sequence coding for at least one peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification in an oilseed plant and a transcriptional termination region is disclosed. The DNA construct may additionally comprise a nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant. The peptide with enzyme activity is preferably a peptide with b-carotene C-4-oxygenase activity, e.g. from the alga *Haematococcus pluvialis*.

Comprised by the invention are also a transgenic oilseed plant cell, e.g. of rape, sunflower, soybean or mustard origin; transgenic oilseed plant-produced xanthophyll; transgenic oilseed plant-produced canthaxanthin; transgenic oilseed plant-produced astaxanthin; and transgenic oilseed plant-produced astaxanthin esters.

3/prts

DNA construct and its use.

The present invention relates to a new DNA construct for transformation into oilseed plants. The DNA construct comprises nucleotide sequences encoding peptides with enzyme activities necessary for the high-level production and esterification of keto group-containing xanthophylls in oilseed plants.

Background of the invention

Carotenoids are produced *de novo* by plants, fungi, algae and some bacteria. A number of biosynthetic steps are needed for the biological production of the carotenoids. There are two chemically different groups of carotenoids, namely carotenes containing only carbon and hydrogen molecules and xanthophylls containing oxygen in the molecule in addition to carbon and hydrogen.

The xanthophylls, and particularly astaxanthin (3,3'-dihydroxy-β-β-carotene-4,4'-dione), are often colored pigments and are used as such or as anti-oxidants.

Carotenes are biological precursors for the production of the oxygen-containing xanthophylls. There are two types of enzymes responsible for the introduction of hydroxy groups and keto groups into the carotenes, namely hydroxylases and ketolases, respectively.

The keto group-containing xanthophyll astaxanthin, which has keto and hydroxy groups, is biosynthetically produced from beta-carotene.

Large-scale production of xanthophylles from natural sources is at present performed by AstaCarotene AB, Gustavsberg, Sweden, by cultivation of the alga *Haematococcus pluvialis* for the production of astaxanthin in esterified form.

It would be desirable to be able to produce keto group-containing xanthophylls particularly astaxanthin, in oilseed plants. Oilseed plants have naturally β-carotene hydroxylases but lack β-carotene C-4-oxygenase enzymes or ketolases.

Description of the invention

The present invention provides DNA constructs enabling and promoting production of keto group containing xanthophylls, especially astaxanthin, in oilseed plants, such as rape, sunflower, soybean and mustard. The DNA construct is transformed into the oilseed plant cell for expression of a protein or fused protein which has an enzyme activity enabling keto group insertion into a carotene or hydroxy carotene for the biosynthetic production of a keto group containing xanthophyll, such as cantaxanthin (β,β-carotene-4,4'-dione) and/or astaxanthin. Use is thus made of the biosynthetic pathway of the oilseed plant to

produce carotenoids. The naturally occurring synthesis of carotenoids involves a number of enzymes, namely 1-D-deoxyxylulose 5-phosphate synthase, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, zeta-carotene desaturase, lycopene beta-cyclase, β-carotene hydroxylase, and β-carotene C-4-oxygenase. Genes coding for peptides having these enzymatic activities may be inserted into the DNA construct of the invention, one or several per construct, to promote high-level production in the transgenic oilseed plant. In case only one enzyme coding gene is inserted per plant, two or more plants may be sexually interbred to produce plants containing all the desired enzyme activities.

Thus, the present invention is directed to a DNA construct comprising in the 5′ to 3′ direction of transcription operably linked a promoter region directing transcription to the seed of an oilseed plant, a nucleotide sequence coding for at least one peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification in an oilseed plant and a transcriptional termination region.

In a preferred embodiment of the invention the DNA construct additionally comprises between the promoter region and the nucleotide sequence coding for at least one peptide with enzyme activity a nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant.

The DNA construct is preferably such that the promoter is a napin promoter, the peptide with enzyme activity necessary for keto group containing xanthophyll production is selected from the group consisting of peptides with 1-D-deoxyxylulose 5-phosphate synthase, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, zeta-carotene desaturase, lycopene beta-cyclase, β-carotene hydroxylase, and β-carotene C-4-oxygenase activity. To promote esterification of astaxanthin a nucleotide sequence coding for a peptide with acyl transferase activity may be included in the group.

In a preferred embodiment of the DNA construct according to the invention the nucleotide sequence coding for a peptide with enzyme activity is a nucleotide sequence coding for a N-terminally truncated β-carotene C-4-oxygenase gene from the alga *Haematococcus pluvialis*.

An example of the DNA construct of the invention is presented in the sequence listing as SEQ ID NO:1 and in Fig.1.

The present invention is also directed to a transgenic oilseed plant cell comprising the DNA construct of the invention, and preferably the oilseed plant is selected from the group consisting of rape, sunflower, soybean and mustard.

The invention is additionally directed to transgenic oilseed plant-produced xanthophyll, e.g. canthaxanthin and astaxanthin.

A preferred aspect of the invention is directed to transgenic oilseed plant-produced astaxanthin esters.

The present invention will now be illustrated with reference to the DNA construct disclosed in the sequence listing and in Fig.1, and the following description of embodiments. However, the invention is not limited to these exemplifications.

Short description of the drawings

Fig.1 illustrates the nucleotide sequence of the DNA construct comprising the napin promoter, the chloroplast localization signal, the N-terminally truncated β-carotene C-4-oxygenase gene and the termination sequence, and the deduced amino acid sequences of the transit peptide and the β-carotene C-4-oxygenase.

Description of embodiments

The invention is illustrated by production of astaxanthin in the seed of oilseed rape. The astaxanthin produced in the seed of the transgenic plant is extracted as part of the extracted oil. By use of conventionally used protocols for Agrobacterium tumefaciens mediated transformation such as described by (Hoekema et al.1983, An et al. 1986, Fry et al. 1987, DeBlock et al. 1988, Radke et al.1988, or Moloney et al. 1989) transgenic plants are produced having a chimeric DNA construct that is genetically inherited and is able to produce astaxanthin. The nucleotide sequence of the chimeric DNA construct consist of four parts of different genetic origin namely: (1) a promoter, (2) a localization signal, (3) a β-carotene C-4-oxygenase coding region and (4) a termination sequence.

The napin promoter directs transcription to the seed of oilseed rape (Stålberg et al 1996). This promoter was coupled to a localization signal similar but not identical to a transit peptide (TP) of Rbcs1a (Krebbers, 1988) that directs the translated product of a fused gene to the chloroplast. The promoter and the TP sequence were ligated to a part of the coding sequence of a ketolase gene BCK (Kajiwara et al. 1995). This enzyme oxygenates β-carotene to canthaxanthin, (Fraser et al. 1997). The chimeric DNA construct was then coupled to a suitable termination sequence, e.g. that of the Agrobacterium tumefaciens nopaline synthase gene (the nos 3′ end)(Bevan et al. 1983), as illustrated in Fig.1.

Cellular storage of Astaxantin

The storage of large amounts of free astaxanthin in plants will be difficult due to toxic effects of the molecule as it intercalates in the plant membranes. An effective esterification of astaxanthin to fatty acids enables storage of the esterified molecules in triacylglycerol containing oleosomes. Thus, an acyl transferase can be claimed to be of fundamental importance for the process, as is proteins that can mediate transport of different forms of astaxanthin from the chloroplast to the vesicles.

Sequences and oligonucleotides used in the construction of the DNA construct

1. Napin promoter (GeneBank ACCESSION No. J02798)

This promoter sequence, a 1145 base pair fragment including the 5' leader sequence has a unique HindIII site at the 5' end. The 3' end was synthesized with an additionally 6 nucleotide BamHI site.

2. Transit peptide similar to RBCS1a (GeneBank ACCESSION No. X13611, X14565)

The transit peptide (TP) was amplified by PCR from –28 to the end of the transit cleavage aa=54/55 site of the Rbcs1a gene. The 5' end was synthesized with a BamHI site and similarly the 3' sequence was synthesized with a XbaI site. The two following oligonucleotides were used for the PCR amplification.

BamHI

5' primer: TP1 5'AGAC **GGATCC** TCAGTCACACAAAGAGTA 3'

SacI XbaI

3' primer: TP2 5'GTTC **GAGCTC TCTAGA** CATGCAGTTAACGC 3'

3. BCK (β-carotene C-4 oxygenase) (Genebank ACCESSION No. D45881)

The BCK fragment was amplified by PCR including a 5' XbaI site and was ligated to the TP already described. The 5' primer (BCK1) used for PCR, is homologous to the BCK sequence from nucleotide 264 and the 3' oligonucleotide (Ax40) ends with a stop codon and was synthesized with a SacI restriction site for cloning. The synthesized fragment was fused to the TP as shown in Fig 1.

Oligonucleotides used for PCR:

XbaI

5' primer: BCK1 5'ACAG **TCTAGA** ATGCCATCCGAGTCGTCA 3'

SacI

3'primer: AX40 5'CACC**GAGCTC**CATGACACTCTTGTGCAGA 3'

Description of SEQ ID NO:1 and SEQ ID NO:2

The sequences shown i Fig.1 are the same as the two sequences which are shown in the sequence listing.

The SEQ ID NO:1 is a nucleotide sequence composed of the following features:

	Nucleotide No.
Cloning site HindIII	1-6
Napin Promoter	1-1145
Cloning site BamHI	1146-1151
Transit peptide leader	1152-1178
Transit peptide coding	1179-1347
Cloning site XbaI	1348-1353
β-carotene C-4-oxygenase	1354-2217
β-carotene C-4-oxygense 3' untranslated	2218-2266
Cloning site SacI	2267-2272
Nopaline synthetase termination	2273-2536
Cloning site EcoRI	2538-2543

The SEQ ID NO: 2 is a deduced amino acid sequence of the fusion protein of the transit peptide and the peptide with β-carotene C-4-oxygenase activity.

References

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Pua E-C, Mehra-Palta A, Nagy F and Chua N-H, (1987). Transgenic plants of Brassica napus. Biotechnology vol 5, 815-817.

Stålberg K, Ellerstöm M, Ezcurra I, Ablov S, Rask L (1996). Disruption of an overlapping E-box/ABRE motif abolished high transcription of the napA storage-protein promoter in transgenic Brassica napus seeds. Planta 199(4):515-9.

Claims

1. Transgenic oilseed plant cell containing a DNA construct comprising in the 5′ to 3′ direction of transcription operably linked a promoter region directing transcription to the seed of the oilseed plant, a nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant, a 5'-truncated beta-carotene C-4-oxygenase gene from the alga *Haematococcus pluvialis* and a transcriptional termination region.

2. Transgenic oilseed plant cell according to claim 1, wherein the cell additionally contains at least one DNA construct selected from DNA constructs comprising in the 5′ to 3′ direction of transcription operably linked a promoter region directing transcription to the seed of the oilseed plant, a nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant, a nucleotide sequence coding for at least one peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification in the oilseed plant and a transcriptional termination region.

3. Transgenic oilseed plant cell according to claim 1 or 2, wherein the promoter is a napin promoter, the peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification is selected from the group consisting of peptides with, 1-D-deoxyxylulose 5-phosphate synthase, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, zeta-carotene desaturase, lycopene beta-cyclase, β-carotene hydroxylase, and acyl transferase activity.

4. Transgenic oilseed plant cell according to claim 1, wherein the nucleotide sequence of the DNA construct is SEQ ID NO:1.

5. Transgenic oilseed plant cell according to any one of claims 1 - 5, wherein the oilseed plant is selected from the group consisting of rape, sunflower, soybean and mustard.

6. Transgenic oilseed plant cell according to any one of claims 1 – 5, wherein the cell expresses xanthophylls.

7. Transgenic oilseed plant cell according to claim 6, wherein a xanthophyll is canthaxanthin.

8. Transgenic oilseed plant cell according to claim 6, wherein a xanthophyll is astaxanthin.

9. Transgenic oilseed plant cell according to claim 8, wherein the astaxanthin comprises astaxanthin esters.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/20011 A1

(54) Title: DNA CONSTRUCT AND ITS USE

(57) Abstract: A DNA construct comprising in the 5' to 3' direction of transcription operably linked a promoter region directing transcription to the seed of an oilseed plant, a nucleotide sequence coding for at least one peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification in an oilseed plant and a transcriptional termination region is disclosed. The DNA construct may additionally comprise a nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant. The peptide with enzyme activity is preferably a peptide with β-carotene C-4-oxygenase activity, e.g. from the alga *<aematococcus pluvialis*. Comprised by the invention are also a transgenic oilseed plant cell, e.g. of rape, sunflower, soybean or mustard origin, and a transgenic oilseed plant-produced xanthophyll, such as canthaxanthin or astaxanthin, and also astaxanthin esters.

Napin promoter

AAGCTTTCTTCATCGGTGATTGATTCCTTTAAAGACTTATGTTTCTTATCTTGCTTCTGA

GGCAAGTATTCAGTTACCAGTTACCACTTATATTCTGGACTTTCTGACTGCATCCTCATT

TTTCCAACATTTTAAATTTCACTATTGGCTGAATGCTTCTTCTTTGAGGAAGAAACAATT

CAGATGGCAGAAATGTATCAACCAATGCATATATACAAATGTACCTCTTGTTCTCAAAAC

ATCTATCGGATGGTTCCATTTGCTTTGTCATCCAATTAGTGACTACTTTATATTATTCAC

TCCTCTTTATTACTATTTTCATGCGAGGTTGCCATGTACATTATATTTGTAAGGATTGAC

GCTATTGAGCGTTTTTCTTCAATTTTCTTTATTTTAGACATGGGTATGAAATGTGTGTTA

GAGTTGGGTTGAATGAGATATACGTTCAAGTGAAGTGGCATACCGTTCTCGAGTAAGGAT

GACCTACCCATTCTTGAGACAAATGTTACATTTTAGTATCAGAGTAAAATGTGTACCTAT

AACTCAAATTCGATTGACATGTATCCATTCAACATAAAATTAAACCAGCCTGCACCTGCA

TCCACATTTCAAGTATTTTCAAACCGTTCGGCTCCTATCCACCGGGTGTAACAAGACGGA

TTCCGAATTTGGAAGATTTTGACTCAAATTCCCAATTTATATTGACCGTGACTAAATCAA

CTTTAACTTCTATAATTCTGATTAAGCTCCCAATTTATATTCCCAACGGCACTACCTCCA

AAATTTATAGACTCTCATCCCCTTTTAAACCAACTTAGTAAACGTTTTTTTTTTTTAATTT

TATGAAGTTAAGTTTTTACCTTGTTTTTAAAAAGAATCGTTCATAAGATGCCATGCCAGA

ACATTAGCTACACGTTACACATAGCATGCAGCCGCGGAGAATTGTTTTTCTTCGCCACTT

GTCACTCCCTTCAAACACCTAAGAGCTTCTCTCTCACAGCACACACATACAATCACATGC

GTGCATGCATTATTACACGTGATCGCCATGCAAATCTCCTTTATAGCCTATAAATTAACT

CATCCGCTTCACTCTTTACTCAAACCAAAACTCATCAATACAAACAAGATTAAAAACATA

End -28 untranslated leader TP start

CACGAGGATCCTCAGTCACACAAAGAGTAAAGAAGAACAATGGCTTCCTCTATGCTCTCT

M A S S M L S

TCCGCTACTATGGTTGCCTCTCCGGCTCAGGCCACTATGGTCGCTCCTTTCAACGGACTT

S A T M V A S P A Q A T M V A P F N G L

AAGTCCTCCGCTGCCTTCCCAGCCACCCGCAAGGCTAACAACGACATTACTTCCATCACA

K S S A A F P A T R K A N N D I T S I T

FIG.1

TP End C-4-Oxygenase

```
AGCAACGGCGGACGCGTTAACTGCATGTCTAGAATGCCATCCGAGTCGTCAGACGCAGCT
 S  N  G  G  R  V  N  C  M  S  R  M  P  S  E  S  S  D  A  A

CGTCCTGCGCTAAAGCACGCCTACAAACCTCCAGCATCTGACGCCAAGGGCATCACGATG
 R  P  A  L  K  H  A  Y  K  P  P  A  S  D  A  K  G  I  T  M

GCGCTGACCATCATTGGCACCTGGACCGCAGTGTTTTTACACGCAATATTTCAAATCAGG
 A  L  T  I  I  G  T  W  T  A  V  F  L  H  A  I  F  Q  I  R

CTACCGACATCCATGGACCAGCTTCACTGGTTGCCTGTGTCCGAAGCCACAGCCCAGCTT
 L  P  T  S  M  D  Q  L  H  W  L  P  V  S  E  A  T  A  Q  L

TTGGGCGGAAGCAGCAGCCTACTGCACATCGCTGCAGTCTTCATTGTACTTGAGTTCCTG
 L  G  G  S  S  S  L  L  H  I  A  A  V  F  I  V  L  E  F  L

TACACTGGTCTATTCATCACCACACATGACGCAATGCATGGCACCATAGCTTTGAGGCAC
 Y  T  G  L  F  I  T  T  H  D  A  M  H  G  T  I  A  L  R  H

AGGCAGCTCAATGATCTCCTTGGCAACATCTGCATATCACTGTACGCCTGGTTTGACTAC
 R  Q  L  N  D  L  L  G  N  I  C  I  S  L  Y  A  W  F  D  Y

AGCATGCTGCATCGCAAGCACTGGGAGCACCACAACCATACTGGCGAAGTGGGGAAAGAC
 S  M  L  H  R  K  H  W  E  H  H  N  H  T  G  E  V  G  K  D

CCTGACTTCCACAAGGGAAATCCCGGCCTTGTCCCCTGGTTCGCCAGCTTCATGTCCAGC
 P  D  F  H  K  G  N  P  G  L  V  P  W  F  A  S  F  M  S  S

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 Y  M  S  L  W  Q  F  A  R  L  A  W  W  A  V  V  M  Q  M  L

GGGGCGCCCATGGCAAATCTCCTAGTCTTCATGGCTGCAGCCCCAATCTTGTCAGCATTC
 G  A  P  M  A  N  L  L  V  F  M  A  A  A  P  I  L  S  A  F

CGCCTCTTCTACTTCGGCACTTACCTGCCACACAAGCCTGAGCCAGGCCCTGCAGCAGGC
 R  L  F  Y  F  G  T  Y  L  P  H  K  P  E  P  G  P  A  A  G

TCTCAGGTGATGGCCTGGTTCAGGGCCAAGACAAGTGAGGCATCTGATGTGATGAGTTTC
 S  Q  V  M  A  W  F  R  A  K  T  S  E  A  S  D  V  M  S  F

CTGACATGCTACCACTTTGACCTGCACTGGGAGCACCACAGATGGCCCTTTGCCCCCTGG
 L  T  C  Y  H  F  D  L  H  W  E  H  H  R  W  P  F  A  P  W
```

C-4 oxygenase Stop

```
TGGCAGCTGCCCCACTGCCGCCGCCTGTCCGGGCGTGGCCTGGTGCCTGCCTTGGCATGA
 W  Q  L  P  H  C  R  R  L  S  G  R  G  L  V  P  A  L  A  *
```

FIG.1 (cont.)

C-4 oxygenase untranslated region Nos term

CCTGGTCCCTCCGCTGGTGACCCAGCGTCTGCACAAGAGTGTCATGGAGCTCGAATTTCC

CCGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTG

CGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAAT

GCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAAT

ACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCAT

end

CTATGTTACTAGATCGGGAATTC

Fig.1 (cont.)

ATTORNEY/DOCKET NO: **HOGL3001/REF**

DECLARATION FOR PATENT APPLICATION AND APPOINTMENT OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention (Design, if applicable) entitled:

DNA CONSTRUCT AND ITS USE

the specification of which (check one):

☐ is attached hereto, or ☒ was filed on: **13 September 2000** as PCT International Application Number **PCT/SE00/01767**

and (if applicable) was amended on:

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in *Title 37, Code of Federal Regulations, §1.56*. I hereby claim foreign priority benefits under *Title 35, United States Code §119* of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATION(S)			PRIORITY CLAIMED	
Number	Country	Day/Month/Year Filed	Yes	No
9903336-7	Sweden	17 September 1999	X	

☐ Additional Priority Application(s) Listed on Following Page(s)

I HEREBY CLAIM THE BENEFIT UNDER TITLE 35 U.S. CODE §119(E) OF ANY U.S. PROVISIONAL APPLICATIONS LISTED BELOW.	
Application Number	Day/Month/Year Filed

☐ Additional Provisional Application(s) Listed on Following Page(s)

I hereby claim the benefit under *Title 35, United States Code, §120* of any United States application(s) or PCT international application(s) designating The United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of *Title 35, United States Code, §112*, I acknowledge the duty to disclose information which is material to patentability as defined in *Title 37, Code of Federal Regulations, §1.56* which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Application Number	Filing Date	Status - Patented, Pending or Abandoned

☐ Additional US/PCT Priority Application(s) listed on Following Page(s)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under *section 1001 of title 18 of the United States Code* and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I (We) hereby appoint as my (our) attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: J. Ernest Kenney, Reg. No. 19,179; Eugene Mar, Reg. No. 25,893; Richard E. Fichter, Reg. No. 26,382; Thomas J. Moore, Reg. No. 28,974; Joseph DeBenedictis, Reg. No. 28,502; Benjamin E. Urcia, Reg. No. 33,805; and

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625 Slaters Lane - 4th Floor
Alexandria, VA 22314-1176

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☒ *See following page(s) for additional joint inventors.*

(04AUG1998)

10070412 .031502

ATTORNEY/DOCKET NO: HOGL3001/REF

CONTINUATION OF DECLARATION FOR PATENT APPLICATION AND APPOINTMENT OF ATTORNEY

Page 2

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FULL NAME OF JOINT INVENTOR	CITIZENSHIP
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DATE	SIGNATURE

FULL NAME OF JOINT INVENTOR	CITIZENSHIP
RESIDENCE ADDRESS	POST OFFICE ADDRESS IS THE SAME AS RESIDENCE ADDRESS UNLESS OTHERWISE SHOWN BELOW
DATE	SIGNATURE

FULL NAME OF JOINT INVENTOR	CITIZENSHIP
RESIDENCE ADDRESS	POST OFFICE ADDRESS IS THE SAME AS RESIDENCE ADDRESS UNLESS OTHERWISE SHOWN BELOW
DATE	SIGNATURE

☐ *See following pages for additional joint inventors/priority applications.*

(04AUG1998)

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coding sequence + termination sequence

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tttccaacat tttaaatttc actattggct gaatgcttct tctttgagga agaaacaatt 180

cagatggcag aaatgtatca accaatgcat atatacaaat gtacctcttg ttctcaaaac 240

atctatcgga tggttccatt tgctttgtca tccaattagt gactacttta tattattcac 300

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                                          Met Ala Ser Ser Met
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Leu Ser Ser Ala Thr Met Val Ala Ser Pro Ala Gln Ala Thr Met Val
                10                  15                  20

gct cct ttc aac gga ctt aag tcc tcc gct gcc ttc cca gcc acc cgc    1290
Ala Pro Phe Asn Gly Leu Lys Ser Ser Ala Ala Phe Pro Ala Thr Arg
            25                  30                  35

aag gct aac aac gac att act tcc atc aca agc aac ggc gga cgc gtt    1338
Lys Ala Asn Asn Asp Ile Thr Ser Ile Thr Ser Asn Gly Gly Arg Val
        40                  45                  50

aac tgc atg tct aga atg cca tcc gag tcg tca gac gca gct cgt cct    1386
Asn Cys Met Ser Arg Met Pro Ser Glu Ser Ser Asp Ala Ala Arg Pro
    55                  60                  65

gcg cta aag cac gcc tac aaa cct cca gca tct gac gcc aag ggc atc    1434
Ala Leu Lys His Ala Tyr Lys Pro Pro Ala Ser Asp Ala Lys Gly Ile
70                  75                  80                  85

acg atg gcg ctg acc atc att ggc acc tgg acc gca gtg ttt tta cac    1482
Thr Met Ala Leu Thr Ile Ile Gly Thr Trp Thr Ala Val Phe Leu His
                90                  95                  100

gca ata ttt caa atc agg cta ccg aca tcc atg gac cag ctt cac tgg    1530
Ala Ile Phe Gln Ile Arg Leu Pro Thr Ser Met Asp Gln Leu His Trp
            105                 110                 115

ttg cct gtg tcc gaa gcc aca gcc cag ctt ttg ggc gga agc agc agc    1578
Leu Pro Val Ser Glu Ala Thr Ala Gln Leu Leu Gly Gly Ser Ser Ser
        120                 125                 130

cta ctg cac atc gct gca gtc ttc att gta ctt gag ttc ctg tac act    1626
Leu Leu His Ile Ala Ala Val Phe Ile Val Leu Glu Phe Leu Tyr Thr
    135                 140                 145
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Gly Leu Phe Ile Thr Thr His Asp Ala Met His Gly Thr Ile Ala Leu
150                 155                 160                 165

agg cac agg cag ctc aat gat ctc ctt ggc aac atc tgc ata tca ctg   1722
Arg His Arg Gln Leu Asn Asp Leu Leu Gly Asn Ile Cys Ile Ser Leu
                170                 175                 180

tac gcc tgg ttt gac tac agc atg ctg cat cgc aag cac tgg gag cac   1770
Tyr Ala Trp Phe Asp Tyr Ser Met Leu His Arg Lys His Trp Glu His
            185                 190                 195

cac aac cat act ggc gaa gtg ggg aaa gac cct gac ttc cac aag gga   1818
His Asn His Thr Gly Glu Val Gly Lys Asp Pro Asp Phe His Lys Gly
        200                 205                 210

aat ccc ggc ctt gtc ccc tgg ttc gcc agc ttc atg tcc agc tac atg   1866
Asn Pro Gly Leu Val Pro Trp Phe Ala Ser Phe Met Ser Ser Tyr Met
    215                 220                 225

tcc ctg tgg cag ttt gcc cgg ctg gca tgg tgg gca gtg gtg atg caa   1914
Ser Leu Trp Gln Phe Ala Arg Leu Ala Trp Trp Ala Val Val Met Gln
230                 235                 240                 245

atg ctg ggg gcg ccc atg gca aat ctc cta gtc ttc atg gct gca gcc   1962
Met Leu Gly Ala Pro Met Ala Asn Leu Leu Val Phe Met Ala Ala Ala
                250                 255                 260

cca atc ttg tca gca ttc cgc ctc ttc tac ttc ggc act tac ctg cca   2010
Pro Ile Leu Ser Ala Phe Arg Leu Phe Tyr Phe Gly Thr Tyr Leu Pro
            265                 270                 275

cac aag cct gag cca ggc cct gca gca ggc tct cag gtg atg gcc tgg   2058
His Lys Pro Glu Pro Gly Pro Ala Ala Gly Ser Gln Val Met Ala Trp
        280                 285                 290

ttc agg gcc aag aca agt gag gca tct gat gtg atg agt ttc ctg aca   2106
Phe Arg Ala Lys Thr Ser Glu Ala Ser Asp Val Met Ser Phe Leu Thr
    295                 300                 305

tgc tac cac ttt gac ctg cac tgg gag cac cac aga tgg ccc ttt gcc   2154
Cys Tyr His Phe Asp Leu His Trp Glu His His Arg Trp Pro Phe Ala
310                 315                 320                 325

ccc tgg tgg cag ctg ccc cac tgc cgc cgc ctg tcc ggg cgt ggc ctg   2202
Pro Trp Trp Gln Leu Pro His Cys Arg Arg Leu Ser Gly Arg Gly Leu
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Val Pro Ala Leu Ala
            345

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Gln Ala Thr Met Val Ala Pro Phe Asn Gly Leu Lys Ser Ser Ala Ala
20 25 30

Phe Pro Ala Thr Arg Lys Ala Asn Asn Asp Ile Thr Ser Ile Thr Ser
35 40 45

Asn Gly Gly Arg Val Asn Cys Met Ser Arg Met Pro Ser Glu Ser Ser
50 55 60

Asp Ala Ala Arg Pro Ala Leu Lys His Ala Tyr Lys Pro Pro Ala Ser
65 70 75 80

Asp Ala Lys Gly Ile Thr Met Ala Leu Thr Ile Ile Gly Thr Trp Thr
85 90 95

Ala Val Phe Leu His Ala Ile Phe Gln Ile Arg Leu Pro Thr Ser Met
100 105 110

Asp Gln Leu His Trp Leu Pro Val Ser Glu Ala Thr Ala Gln Leu Leu
115 120 125

Gly Gly Ser Ser Ser Leu Leu His Ile Ala Ala Val Phe Ile Val Leu
130 135 140

Glu Phe Leu Tyr Thr Gly Leu Phe Ile Thr Thr His Asp Ala Met His
145 150 155 160

Gly Thr Ile Ala Leu Arg His Arg Gln Leu Asn Asp Leu Leu Gly Asn
165 170 175

Ile Cys Ile Ser Leu Tyr Ala Trp Phe Asp Tyr Ser Met Leu His Arg
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Lys His Trp Glu His His Asn His Thr Gly Glu Val Gly Lys Asp Pro
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Asp Phe His Lys Gly Asn Pro Gly Leu Val Pro Trp Phe Ala Ser Phe
210 215 220

Met Ser Ser Tyr Met Ser Leu Trp Gln Phe Ala Arg Leu Ala Trp Trp
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Ala Val Val Met Gln Met Leu Gly Ala Pro Met Ala Asn Leu Leu Val
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Phe Met Ala Ala Ala Pro Ile Leu Ser Ala Phe Arg Leu Phe Tyr Phe
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        275                 280                 285

Gln Val Met Ala Trp Phe Arg Ala Lys Thr Ser Glu Ala Ser Asp Val
    290                 295                 300

Met Ser Phe Leu Thr Cys Tyr His Phe Asp Leu His Trp Glu His His
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cagatggcag aaatgtatca accaatgcat atatacaaat gtacctcttg ttctcaaaac 240

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acattagcta cacgttacac atagcatgca gccgcggaga attgtttttc ttcgccactt 960

gtcactccct tcaaacacct aagagcttct ctctcacagc acacacatac aatcacatgc 1020

gtgcatgcat tattacacgt gatcgccatg caaatctcct ttatagccta taaattaact 1080

catccgcttc actctttact caaaccaaaa ctcatcaata caaacaagat taaaaacata 1140

cacgaggatc ctcagtcaca caaagagtaa agaagaaca atg gct tcc tct atg    1194
                                          Met Ala Ser Ser Met
                                            1               5

ctc tct tcc gct act atg gtt gcc tct ccg gct cag gcc act atg gtc   1242
Leu Ser Ser Ala Thr Met Val Ala Ser Pro Ala Gln Ala Thr Met Val
                10                  15                  20

gct cct ttc aac gga ctt aag tcc tcc gct gcc ttc cca gcc acc cgc   1290
Ala Pro Phe Asn Gly Leu Lys Ser Ser Ala Ala Phe Pro Ala Thr Arg
            25                  30                  35

aag gct aac aac gac att act tcc atc aca agc aac ggc gga cgc gtt   1338
Lys Ala Asn Asn Asp Ile Thr Ser Ile Thr Ser Asn Gly Gly Arg Val
        40                  45                  50

aac tgc atg tct aga atg cca tcc gag tcg tca gac gca gct cgt cct   1386
Asn Cys Met Ser Arg Met Pro Ser Glu Ser Ser Asp Ala Ala Arg Pro
    55                  60                  65

gcg cta aag cac gcc tac aaa cct cca gca tct gac gcc aag ggc atc   1434
Ala Leu Lys His Ala Tyr Lys Pro Pro Ala Ser Asp Ala Lys Gly Ile
70                  75                  80                  85

acg atg gcg ctg acc atc att ggc acc tgg acc gca gtg ttt tta cac   1482
Thr Met Ala Leu Thr Ile Ile Gly Thr Trp Thr Ala Val Phe Leu His
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gca ata ttt caa atc agg cta ccg aca tcc atg gac cag ctt cac tgg   1530
Ala Ile Phe Gln Ile Arg Leu Pro Thr Ser Met Asp Gln Leu His Trp
            105                 110                 115

ttg cct gtg tcc gaa gcc aca gcc cag ctt ttg ggc gga agc agc agc   1578
Leu Pro Val Ser Glu Ala Thr Ala Gln Leu Leu Gly Gly Ser Ser Ser
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cta ctg cac atc gct gca gtc ttc att gta ctt gag ttc ctg tac act   1626
Leu Leu His Ile Ala Ala Val Phe Ile Val Leu Glu Phe Leu Tyr Thr
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ggt cta ttc atc acc aca cat gac gca atg cat ggc acc ata gct ttg   1674
Gly Leu Phe Ile Thr Thr His Asp Ala Met His Gly Thr Ile Ala Leu
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agg cac agg cag ctc aat gat ctc ctt ggc aac atc tgc ata tca ctg   1722
Arg His Arg Gln Leu Asn Asp Leu Leu Gly Asn Ile Cys Ile Ser Leu
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tac gcc tgg ttt gac tac agc atg ctg cat cgc aag cac tgg gag cac   1770
Tyr Ala Trp Phe Asp Tyr Ser Met Leu His Arg Lys His Trp Glu His
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cac aac cat act ggc gaa gtg ggg aaa gac cct gac ttc cac aag gga   1818
His Asn His Thr Gly Glu Val Gly Lys Asp Pro Asp Phe His Lys Gly
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aat ccc ggc ctt gtc ccc tgg ttc gcc agc ttc atg tcc agc tac atg   1866
Asn Pro Gly Leu Val Pro Trp Phe Ala Ser Phe Met Ser Ser Tyr Met
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tcc ctg tgg cag ttt gcc cgg ctg gca tgg tgg gca gtg gtg atg caa   1914
Ser Leu Trp Gln Phe Ala Arg Leu Ala Trp Trp Ala Val Val Met Gln
230                 235                 240                 245

atg ctg ggg gcg ccc atg gca aat ctc cta gtc ttc atg gct gca gcc   1962
Met Leu Gly Ala Pro Met Ala Asn Leu Leu Val Phe Met Ala Ala Ala
                250                 255                 260

cca atc ttg tca gca ttc cgc ctc ttc tac ttc ggc act tac ctg cca   2010
Pro Ile Leu Ser Ala Phe Arg Leu Phe Tyr Phe Gly Thr Tyr Leu Pro
            265                 270                 275

cac aag cct gag cca ggc cct gca gca ggc tct cag gtg atg gcc tgg   2058
His Lys Pro Glu Pro Gly Pro Ala Ala Gly Ser Gln Val Met Ala Trp
        280                 285                 290

ttc agg gcc aag aca agt gag gca tct gat gtg atg agt ttc ctg aca   2106
Phe Arg Ala Lys Thr Ser Glu Ala Ser Asp Val Met Ser Phe Leu Thr
    295                 300                 305

tgc tac cac ttt gac ctg cac tgg gag cac cac aga tgg ccc ttt gcc   2154
Cys Tyr His Phe Asp Leu His Trp Glu His His Arg Trp Pro Phe Ala
310                 315                 320                 325

ccc tgg tgg cag ctg ccc cac tgc cgc cgc ctg tcc ggg cgt ggc ctg   2202
Pro Trp Trp Gln Leu Pro His Cys Arg Arg Leu Ser Gly Arg Gly Leu
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gtg cct gcc ttg gca tgacctggtc cctccgctgg tgacccagcg tctgcacaag   2257
Val Pro Ala Leu Ala
            345

agtgtcatgg agctcgaatt tccccgatcg ttcaaacatt tggcaataaa gtttcttaag 2317

attgaatcct gttgccggtc ttgcgatgat tatcatataa tttctgttga attacgttaa 2377

gcatgtaata attaacatgt aatgcatgac gttatttatg agatgggttt ttatgattag 2437

agtcccgcaa ttatacattt aatacgcgat agaaaacaaa atatagcgcg caaactagga 2497

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<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: deduced fusion protein of transit peptide + peptide with beta-carotene C-4 oxygenase activity

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Gln Ala Thr Met Val Ala Pro Phe Asn Gly Leu Lys Ser Ser Ala Ala
20 25 30

Phe Pro Ala Thr Arg Lys Ala Asn Asn Asp Ile Thr Ser Ile Thr Ser
35 40 45

Asn Gly Gly Arg Val Asn Cys Met Ser Arg Met Pro Ser Glu Ser Ser
50 55 60

Asp Ala Ala Arg Pro Ala Leu Lys His Ala Tyr Lys Pro Pro Ala Ser
65 70 75 80

Asp Ala Lys Gly Ile Thr Met Ala Leu Thr Ile Ile Gly Thr Trp Thr
85 90 95

Ala Val Phe Leu His Ala Ile Phe Gln Ile Arg Leu Pro Thr Ser Met
100 105 110

Asp Gln Leu His Trp Leu Pro Val Ser Glu Ala Thr Ala Gln Leu Leu
115 120 125

Gly Gly Ser Ser Ser Leu Leu His Ile Ala Ala Val Phe Ile Val Leu
130 135 140

Glu Phe Leu Tyr Thr Gly Leu Phe Ile Thr Thr His Asp Ala Met His
145 150 155 160

Gly Thr Ile Ala Leu Arg His Arg Gln Leu Asn Asp Leu Leu Gly Asn
165 170 175

Ile Cys Ile Ser Leu Tyr Ala Trp Phe Asp Tyr Ser Met Leu His Arg
180 185 190

Lys His Trp Glu His His Asn His Thr Gly Glu Val Gly Lys Asp Pro
195 200 205

Asp Phe His Lys Gly Asn Pro Gly Leu Val Pro Trp Phe Ala Ser Phe
210 215 220

Met Ser Ser Tyr Met Ser Leu Trp Gln Phe Ala Arg Leu Ala Trp Trp
225 230 235 240

Ala Val Val Met Gln Met Leu Gly Ala Pro Met Ala Asn Leu Leu Val
245 250 255

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Phe Met Ala Ala Ala Pro Ile Leu Ser Ala Phe Arg Leu Phe Tyr Phe
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Gly Thr Tyr Leu Pro His Lys Pro Glu Pro Gly Pro Ala Ala Gly Ser
       275                 280                 285

Gln Val Met Ala Trp Phe Arg Ala Lys Thr Ser Glu Ala Ser Asp Val
   290                 295                 300

Met Ser Phe Leu Thr Cys Tyr His Phe Asp Leu His Trp Glu His His
305                 310                 315                 320

Arg Trp Pro Phe Ala Pro Trp Trp Gln Leu Pro His Cys Arg Arg Leu
                325                 330                 335

Ser Gly Arg Gly Leu Val Pro Ala Leu Ala
           340                 345
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